

This listing of claims will replace all prior versions, and listings, of claims in the application:

### **LISTING OF CLAIMS**

1. (*currently amended*) A method of determining the copy number (CN) of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

- (1) adding to the sample nucleotides, primers, polymerase, probes and optionally, any additional reagents required for amplification;
- (2) performing one or more amplification cycles to amplify the NucSeqI, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII), and the following amplification steps are carried out:
  - (a) NucSeqI is amplified,
  - (b) NucSeqII is amplified,
  - (c) a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI and present in a control sample is amplified at multiple dilutions, and
  - (d) a fourth nucleotide sequence II' (NucSeqII') corresponding to NucSeqII and present in a control sample is amplified at multiple dilutions,

wherein

- (i) the ratio of concentration of NucSeqI' ~~[[and]] to the concentration of NucSeqII' is known,~~
  - (ii) ~~standard curves SC<sub>I</sub> and SC<sub>II</sub> comprising at least two reference points are generated by wherein amplification of [[the]] NucSeqI' and NucSeqII' at multiple dilutions results in the generation of standard curves SC<sub>I</sub> and SC<sub>II</sub>, respectively, at multiple dilutions,~~
  - (iii) ~~at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and~~
  - (iv) ~~NucSeqI' and NucSeqII' are localized on a single vector; and~~
- (3) ~~determining from the results of the amplifications of step (2) such that the concentrations of NucSeqI and NucSeqII are determined by using the respective standard curves SC<sub>I</sub> and SC<sub>II</sub>, to obtain the relative such that copy number-CN of NucSeqI relative with respect to NucSeqII is determined using by the formula;~~

$$\text{relative CN} = \frac{\text{Conc-ISC}_I}{\text{Conc-ISC}_{II}}$$

wherein, in said formula,

- (i) "relative CN" is the ratio of the ~~relative copy number CN~~ of NucSeqI relative to ~~the CN of NucSeqII~~ in the sample;
- (ii) "Conc-ISC<sub>I</sub>" is the concentration of NucSeqI determined ~~using from~~ standard curve SC<sub>I</sub>; and
- (iii) "Conc-ISC<sub>II</sub>" is the concentration of NucSeqII determined ~~using~~ ~~from standard curve SC<sub>II</sub>~~[[, and]]

~~wherein at least one pair of amplification reactions selected from (a) and (b), and (c) and (d) is performed in a single container and monitored spectrophotometrically during amplification, and NucSeqI' and NucSeqII' are localized on a single vector.~~

2. *(currently amended)* A method for determining the absolute CN of a nucleotide sequence NucSeqI in a sample, comprising:

- (a) determining the relative CN using the method of according to claim 18, and  
~~wherein an absolute copy number is determined by~~
- (b) multiplying the relative CN by the absolute CN number of copies of NucSeqII per cell.

3. *(previously presented)* A method according to claim 1, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI sequences are localized on a single vector.

4. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqI and NucSeqI' are the same.

5. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqII and NucSeqII' are the same.

6. *(previously presented)* A method according to claim 2, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

7. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqI and the NucSeqI' are the same.

8. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI' are the same.

9. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqI and the NucSeqI' are the same.

10. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII' are the same.

11. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII' are the same.

12. *(previously presented)* A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII' are the same.

13. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII' are the same.

14. *(previously presented)* A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII' are the same.

15. *(previously presented)* A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.

16. *(previously presented)* A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII' are the same.

17. *(new)* A method according to claim 1, wherein the sample is derived from cells.

18. *(new)* A method according to claim 17, wherein an absolute CN of NucSeqII per cell is known.

19. *(new)* A method according to claim 18, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

20. *(new)* A method according to claim 18, wherein the sequences of NucSeqI and the NucSeqI' are the same.

21. *(new)* A method according to claim 18 wherein the sequences of NucSeqII and the NucSeqII' are the same.

22. *(new)* A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII' are the same.

23. *(new)* A method according to claim 20 wherein the sequences of NucSeqII and the NucSeqII' are the same.

24. *(new)* A method of determining the CN of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

- (1) adding to the sample nucleotides, primers, polymerase, probes and optionally, any additional reagents required for amplification;
- (2) performing one or more amplification cycles to amplify NucSeqI, wherein the sample comprises a second nucleotide sequence II (NucSeqII), and the following amplification steps are carried out:
  - (a) NucSeqI is amplified,
  - (b) NucSeqII is amplified,
  - (c) a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI and present in a control sample is amplified at multiple dilutions, and
  - (d) a fourth nucleotide sequence II' (NucSeqII') corresponding to NucSeqII and present in a control sample is amplified at multiple dilutions,

wherein

- (i) the ratio of the concentration of NucSeqI' to the concentration of NucSeqII' is known,
- (ii) standard curves SC<sub>I</sub> and SC<sub>II</sub> comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions,
- (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and

- (iv) NucSeqI' and NucSeqII' are localized on a single vector; and
- (3) determining from the results of the amplifications of step (2) the concentrations of NucSeqI and NucSeqII using the respective standard curves SC<sub>I</sub> and SC<sub>II</sub>, to obtain the relative CN of NucSeqI with respect to NucSeqII, by the formula:

$$\text{relative CN} = \frac{\text{Conc-I}_{\text{SCI}}}{\text{Conc-II}_{\text{SCII}}}$$

wherein, in said formula,

- (a) "relative CN" is the CN of NucSeqI relative to the CN of NucSeqII in the sample;
- (b) "Conc-I<sub>SCI</sub>" is the concentration of NucSeqI determined from standard curve SC<sub>I</sub>; and
- (c) "Conc-II<sub>SCII</sub>" is the concentration of NucSeqII determined from standard curve SC<sub>II</sub>.